ENVIRONMENTAL HEALTH PROJECT

Activity Report 111

Malaria Vector Studies in Eritrea

by

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Abbreviations

EIR	entomological inoculation rate
EHP	Environmental Health Project
ELISA	enzyme-linked immunosorbent assay
NBC	night biting collection
NDVI	Normalized Difference Vegetation Index
NMCP	National Malaria Control Program
NRS Zone	Northern Red Sea Zone
PCR	polymerase chain reaction
PSC	pyrethrum spray collection
SRS Zone	Southern Red Sea Zone
USAID	U.S. Agency for International Development

Executive Summary

Malaria accounts for over 30% of the total outpatient morbidity in Eritrea, and about 28% of all hospital admissions are malaria related. Almost 67% of the resident population of Eritrea lives in malaria endemic areas. The malaria situation is complicated since the country is especially prone to epidemics, which in the past have been a cause of considerable morbidity and mortality. Although malaria remains a major cause of mortality in the country, little is known about the *Anopheles* mosquito species responsible for transmission of malaria in Eritrea. It also is clear that malaria parasite transmission is driven by the temporal and spatial patterns of vector species of anopheline mosquitoes. Since each mosquito species has a geographical range that is limited according to physiologic levels of tolerance to environmental conditions, understanding how the degree of ecological diversity and biotic interactions would be critical in determining how vector populations are structured.

In Eritrea, larval control is implemented as part of an integrated approach to malaria control. However, for larval control to be an integral part of a vector management program, a sound understanding of the factors responsible for larval production of the principal vectors of malaria is crucial. On this basis, the NMCP initiated studies on the spatial patterns of anopheline species and larval ecology in Eritrea with the overall goal of providing insights into the bionomics of malaria parasite vectors.

In this report, the results of the first detailed information on the spatial distribution, vector bionomics and larval ecology of the anopheline species in Eritrea is reported.

The importance of the information generated from this study for the development of ecologically sensitive and efficient mosquito control strategies that would guide decisions on vector control operations cannot be overstated.

1 Spatial Distribution of Malaria Vectors in Eritrea

1.1. Introduction

1.1.1. Country Profile

Eritrea is situated in the horn of Africa and lies between 16°30' and 43°20' east longitude and between 12°42' and 18°2' north latitude. It is bordered by Sudan to the north and northwest, Ethiopia to the south, Djibouti to the southeast and the Red Sea to the east. Its area is approximately 124,000 square kilometers, including the Dahlak Archipelago and the islands in the Red Sea. Rainfall is scanty and highly seasonal; the annual average ranges from 400–650 mm in the highlands and from 200–300 mm in the lowlands. The country is divided into six administrative regions, referred to as zones: Anseba, Debub, Gash-Barka, Maakel, Northern Red Sea (NRS) and Southern Red Sea (SRS) (see Figure 1). The total population is about 3.5 million with an annual growth rate of 3%. Malaria accounts for 30% of the total outpatient morbidity and about 28% of all hospital admissions. *Plasmodium falciparum* is the most prevalent (94%) parasite species.

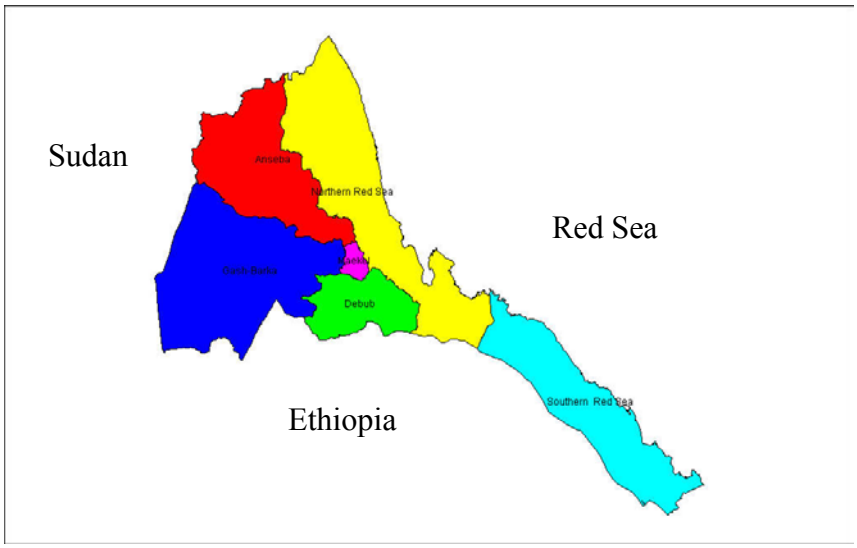


Figure 1. Map of Eritrea, showing administrative boundaries

1.1.2. Malaria Vector Studies

Mosquitoes (Diptera: Culicidae) show limited geographical range because each vector species can survive only under certain optimal environmental conditions. Temperature, rainfall, relative humidity and altitude are the four major factors affecting the presence and abundance of anopheline mosquitoes in a given area. The quality of breeding sites and their distribution have a direct bearing on mosquito population. Physical factors (such as water temperature, light, water movement, wave action, vegetation, hydrogen, ion concentration, soil type and salinity) and biotic interactions (such as predation) are known to influence mosquito species assemblages. Mosquitoes often dominate in wetland ecosystems where suitable breeding sites are abundant and other physical factors are optimal for adult survival. The degree of spatial heterogeneity and biotic interactions play an important role in determining how mosquito populations are structured.

Knowledge of the spatial and temporal patterns of anopheline species provides insights into the dynamics of malaria transmission and therefore can result in efficient implementation of control operations. It is against this background that the National Malaria Control Program (NMCP) in Eritrea, with the technical support from the Environmental Health Project (EHP) of the U.S. Agency for International Development (USAID), undertook a step to characterize vector distributions in the country. Most information on anopheline species and ecology was basically extrapolation from work conducted under the Ethiopian regime. The overall objective of the survey was to determine the species composition, distribution patterns and relative abundance of malaria vectors in the country. The information generated from the distribution survey of vector species provides an understanding of species assemblage patterns and gives a clue to the spatial extent of the disease. This should help form a basis for future integrated programs for malaria control in the country.

1.2. Methods

The survey was undertaken in two phases, one in 1999–2000 and the second in 2000–2001, corresponding to the peak malaria transmission season in the country. In the first and second phases of the study, a total of 170 villages and 135 villages were sampled, respectively. At least three villages were selected randomly from each subzone in the country. Indoor resting anopheline mosquitoes were sampled by pyrethrum spray collection (PSC) from 6:00 a.m.–8:00 a.m. in ten randomly selected houses in each village. All female anopheline mosquitoes were preserved on moist cotton and later identified to species, using morphological criteria. The mosquito specimens were then preserved on Drierite/silica gel for further processing. The specimens were cross-checked for correct identification at the NMCP headquarters.

1.3. Results

1.3.1. Vector Species Abundance

In the first phase of the study a total of 1,139 anopheline species were collected. *Anopheles gambiae*[1] was the most abundant species, forming 75.6% (n = 861) of the total anophelines collected. *An. d'thali* was the second most abundant species (18%, n = 209). Other species collected included *An. cinereus, An. squamosus, An. rupiculos, An. harperi, An. demeilloni* and *An. rhodesiensis,* though in very low densities (Table 1). Of the total number of anophelines collected (n = 1,374) in the second phase of the study, 91.9% were *An. gambiae* (Table 2). Over the whole sampling period a total of 13 anopheline species were collected. Overall, *An. gambiae* was the predominant species (Fig 1b). This suggests that this species, under appropriate conditions of temperature and humidity, and the presence of infectious gametocyte pool in the population, forms a major vector of malaria. The rest of the species form only a small proportion of the total anophelines collected.

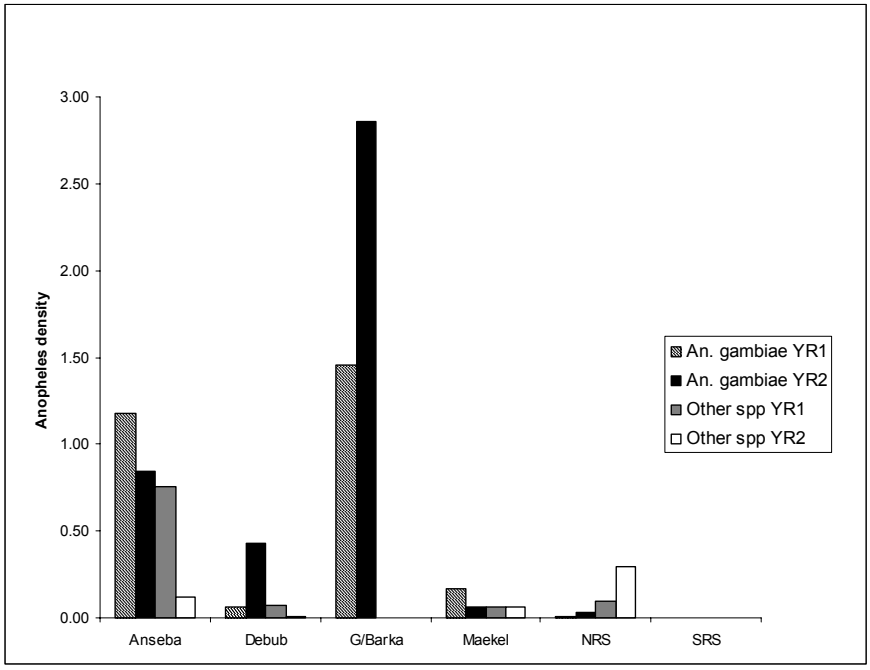


Figure 1b. Density of anopheline species sampled in year 1 and year 2

The results also show that there is a great diversity of anopheline fauna in the country. A much greater diversity in anopheline species is present in Anseba and Debub zones, and this can be attributed to the diversity in ecological prototypes in the two zones.

[1] *An. gambiae* is a complex of six sibling species (see Section 1.3.2).

Table 1. Distribution of *Anopheles* Malaria Vectors in Eritrea (Year 1)

Zone	Anseba	Debub	Gash-Barka	NRS	SRS	Maekel	Total
# Villages Surveyed	30	30	30	30	17	30	**167**
# Houses Sampled	300	300	300	300	169	300	**1,669**
An. Gambiae	353	18	438	49	0	3	**861**
An. Cinereus	6	15	0	0	0	21	**42**
An. d'thali	207	2	0	0	0	0	**209**
An. squamosus	0	0	0	0	0	8	**8**
An. rhodesiensis	0	1	0	0	0	0	**1**
An. rupicolus	10	0	0	0	0	0	**10**
An. Harperi	0	0	1	0	0	0	**1**
An. demeilloni	3	1	0	0	0	0	**4**
An. garnhami	0	3	0	0	0	0	**3**
Total	**579**	**40**	**439**	**49**	**0**	**32**	**1,139**

Significantly more anopheline mosquitoes were collected in Gash-Barka (51.3%, n=1295) and Anseba zones (34.5%, n= 867). The indoor household density in Gash-Barka and Anseba was 2.2 and 1.5 anophelines per household, respectively. Very low numbers of mosquitoes were sampled in Debub, Maekel and NRS over the two phases of the study. No adult anophelines were sampled in the SRS zone from indoor collections. Overall, the difference in *Anopheles* densities over the two years was not significant ($F_{5, 3018} = 2.42$, P=0.119).

Table 2. Distribution of *Anopheles* Malaria Vectors in Eritrea (Year 2)

Zone	Anseba	Debub	Gash-Barka	NRS	Maekel	Total
# Villages Surveyed	30	30	30	30	15	**135**
# Houses Sampled	300	300	300	300	150	**1,350**
An. Gambiae	253	129	856	20	5	**1,263**
An. Cinereus	6	9	0	0	27	**42**
An. D'thali	9	0	0	17	0	**26**
An. Squamosus	0	0	0	0	12	**12**
An. Rhodesiensis	19	0	0	0	0	**19**
An. Rupicolus	1	0	0	0	1	**2**
An. Funestus	0	2	0	0	0	**2**
An. Chrysti	0	0	0	0	3	**3**
An. Welcomi	0	1	0	3	0	**4**
An. Pharoensis	0	0	0	0	1	**1**
Total	**288**	**141**	**856**	**40**	**49**	**1,374**

A total of 80 anopheline mosquitoes were collected in Maekel zone in the central highlands (Table 3). The presence of anophelines at very high altitudes (>1800 m) is an indication that with change in environmental conditions such highland zones will no longer be safe from malaria.

Table 3: Densities of Anopheline Mosquitoes in Eritrea

Zone	Total An.	*Anopheles* density	# Houses	% Total	Std. Deviation	95% CI	
Anseba	867	1.45	600	34.5	4.99	–8.3	11.2
Debub	181	0.30	600	7.2	2.99	–5.6	6.2
Gash-Barka	1,295	2.16	600	51.5	7.86	–13.3	17.6
Maekel	80	0.18	450	3.2	0.92	–1.6	1.9
NRS	90	0.15	600	3.6	1.16	–2.1	2.4
SRS	0	0.00	169	0.0	0.00	0	0

1.3.2. PCR Analysis of *An. gambiae s.l.* Sibling Species

Anopheles gambiae is a complex of closely related species that are morphologically indistinguishable. The complex is made up of six sibling species: *An. gambiae s.s, An. arabiensis, An. merus, An. melas, An. quadriannulatus,* and *An. bwambae.* The sibling species differ greatly in their malaria transmission potential and behavioral patterns. Due to variations in microclimatic conditions, the sibling species composition of the *An. gambiae* complex may differ within a given area. Therefore, precise identification of *An. gambiae* and other species complexes to determine the vectorial system is central to successful malaria control.

In order to characterize the *An. gambiae* species sampled, a total of 1,446 specimens was analyzed by Polymerase Chain Reaction (PCR). Out of the total positively identified, over 99% (n= 1309) were identified as *An. arabiensis.* This is important because it suggests that control operations can be targeted to a narrow range of the vector system. An in-depth understanding of the bionomics of this species remains critical for the successful control of malaria. So far, two-year data on temporal and behavior patterns have been generated and will answer important operational questions.

Although *An. arabiensis* is generally known to be more zoophilic[2] and exophilic[3], it remains an important vector of malaria in less humid and drier ecotypes. Less than 1% (n=1) of the mosquitoes were identified as *An. gambiae s.s.* The data so far indicate that *An. arabiensis* forms the main vectorial system in the country.

[2] Zoophily: Tendency to derive blood from animal sources; [3] Exophily: Tendency to rest outdoors.

At least 9.4% (n= 136) of the *An. gambiae* tested could not be identified to species by PCR using the primers specific for *An. gambiae s.s* and *An. arabiensis.* A proportion of the unidentified species could be *An. quadriannulatus;* hence there is a need to test for this sibling species. All 237 larval samples analyzed by PCR were positively identified as *An. arabiensis,* further indicating that this could be the major sibling species of the *An. gambiae* complex present in the country.

1.3.3. Species Distribution

Analysis of the data revealed a significant variation in mosquito densities between zones ($F_{5, 204} = 4.77$, $P < 0.001$). Intrazonal variation explained 90% of the total variation in mosquito distribution. Further, house level variation in mosquito densities was highly significant ($F_{5, 3018} = 21.01$, $P < 0.001$). Much greater variation in indoor resting mosquitoes was seen in Gash-Barka and Anseba zone. In these two zones there is focal distribution of mosquitoes with some villages having low densities, while others show generally high densities (Figure 2). This may be a function of aggregation of mosquitoes within the zone or village. The data show that over 80% of the total anophelines were sampled from less than 20% of the villages (Figure 3a). It is further observed that even within villages the pattern of mosquito distribution is not homogenous. Almost 100% of the total mosquitoes were sampled from only 10% of the houses. This heterogeneity in distribution may have important operational significance for the design of control operations to target villages within zones where there are high densities of mosquitoes. A further step in this approach would be to apply control measures selectively to houses within villages that have greater risk of mosquito infestation. This, however, would require elaborate stratification of houses based on such criteria as closeness to breeding sites, or defining physical characteristics of housing types that attract mosquitoes.

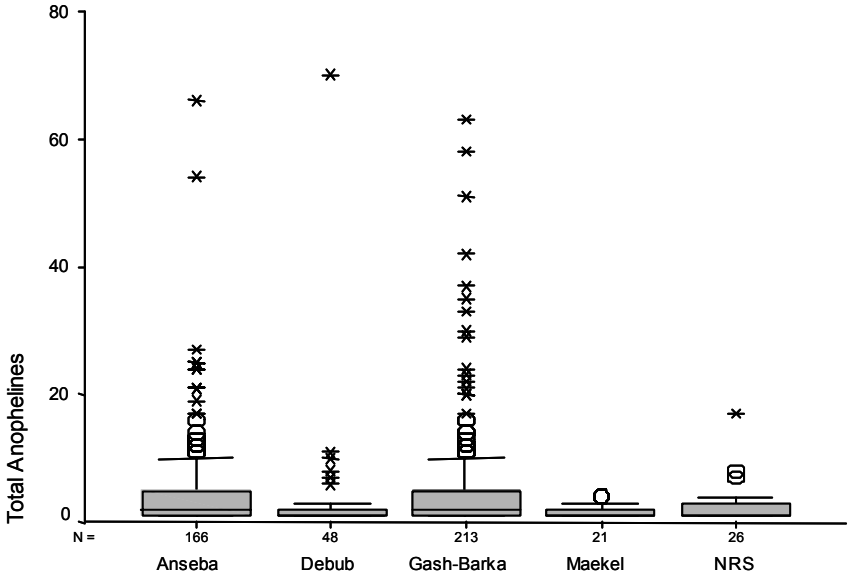


Figure 2. Variation in anopheline densities by village in the five zones

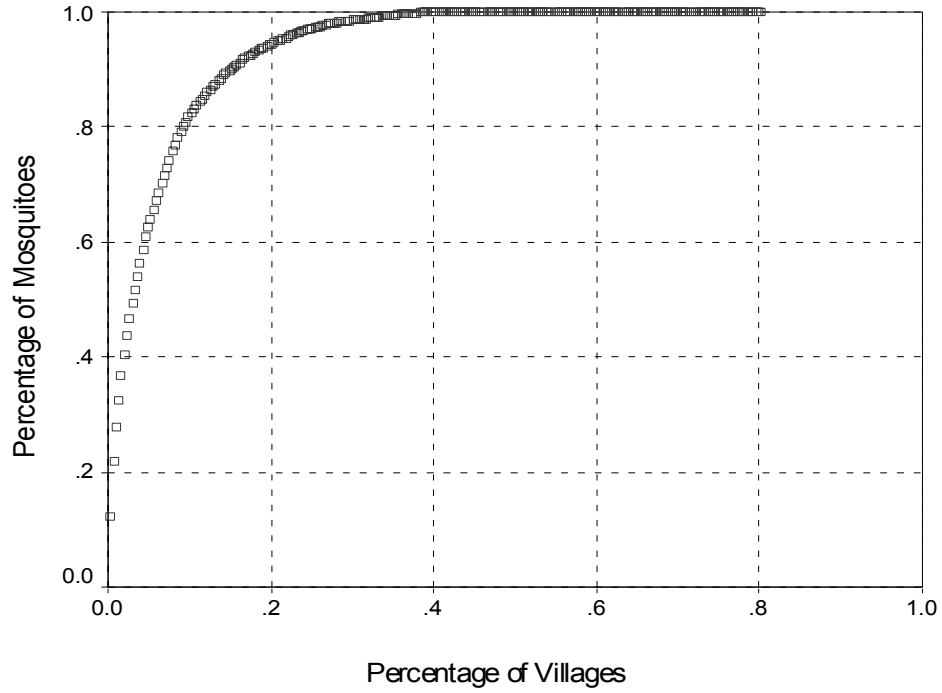


Figure 3a. Spatial aggregation of *Anopheles* species within zones

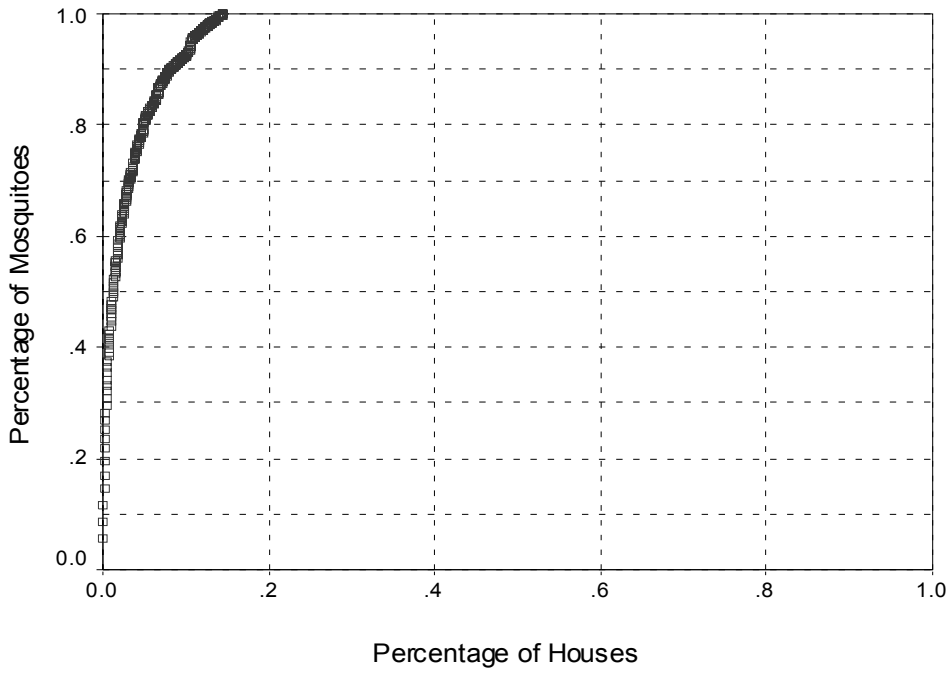


Figure 3b. Spatial aggregation of *Anopheles* species within villages

1.3.4. Variation of Mosquito Species by Altitudinal Zones

Altitude has great influence on temperature and a lesser one on humidity. The selection of villages for the vector distribution study captured most of these altitudinal variations. Analysis of the data generated from the vector distribution survey shows that densities of anopheline mosquitoes tended to increase with a decrease in altitude. Anopheline mosquitoes were present at altitudes greater than 2000 m, indicating that such high altitude areas will not remain malaria-free with current climatic changes (Figure 4). High densities of anophelines were also sampled at altitudes between 1400–1800 m. These are fringe areas that may be prone to malaria epidemics and they pose a major malaria risk. The scenario is complicated by the fact that the country's highest population density is found within this zone. Surveillance mechanisms have to be developed in these areas to monitor vector populations and malaria transmission with a view of averting future epidemics.

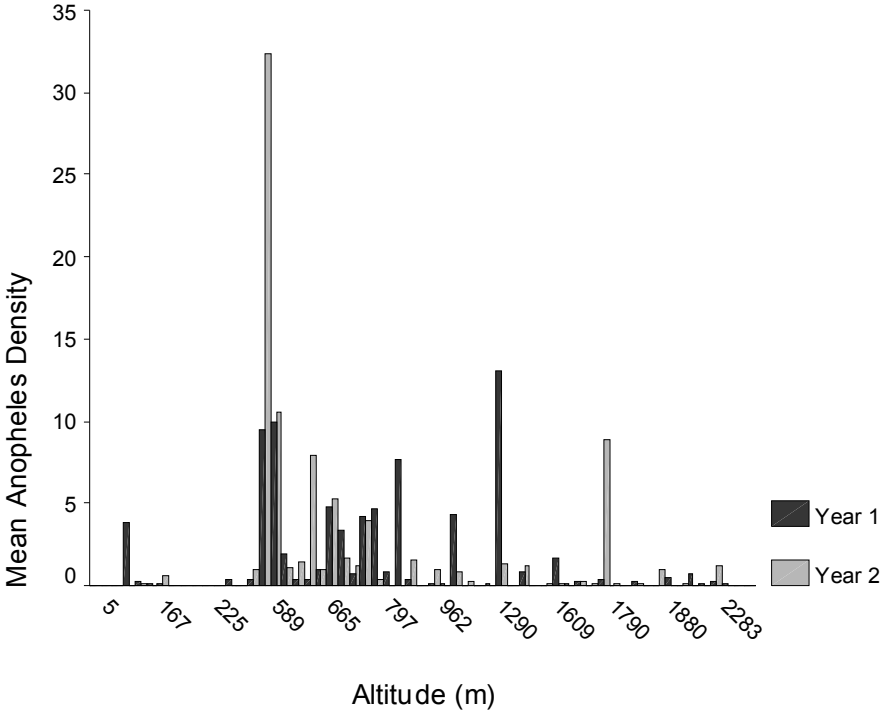


Figure 4. Variation of anopheline mosquitoes by altitude

1.3.5. Type of Housing, Wall Type and *Anopheles* Species Densities

The type of housing was found to be a significant factor in the densities of mosquitoes resting indoors ($F_{3, 3018} = 29.68$, $P < 0.001$). Of the total mosquitoes collected, 80.6% (n= 1936) were sampled from Agudo type dwellings (Table 4). Agudo houses are mainly rounded with walls made of mud and are grass thatched. At least 18.7% (n= 450) of the specimens came from rectangular type housing (Four sided houses with tin roof, with walls made of stone or concrete blocks). The high density of *Anopheles* mosquitoes in Agudo housing shows the degree of predisposition to mosquito bites for individuals living in these dwellings and therefore the substantial risk of malaria infection. Further analysis shows that intradomicile variation accounted for more than 90% of the variability in mosquito densities. This suggests that the presence of mosquitoes is the interplay of multiple factors, such as the presence and proximity to mosquito breeding sites and variations in the physical characteristics of the housing types.

Table 4. Density of Anopheline Mosquitoes in Different Housing Types

House Type	# Houses	Mos. Density	Total *Anopheles*	% of Total *Anopheles*	95 % CI	
Agudo	1,072	1.81	1,936	80.6	1.40	2.21
Hudmo[4]	12	0.00	0	0.0	0.00	0.00
Portable[5]	123	0.13	16	0.7	0.03	0.23
Rectangular	1,812	0.25	450	18.7	0.15	0.34
Total	3,019	0.80	2,402	100	0.64	0.95

Though the data suggest the tendency for variability in mosquito densities based on housing type, over 90% of the Agudo dwellings sampled were from Gash-Barka zone, which had the highest densities of mosquitoes. A sampling design aimed specifically at testing the influence of housing characteristics on mosquito densities and malaria prevalence in each ecological zone would be necessary to pinpoint the source of this variability.

The wall types in the houses sampled were grouped into seven categories: mud, mat, plaster, stone, thatch, tin and wooden (Table 5). Wall type was significant in explaining variation in mosquito densities ($F_{7, 3010} = 4.038$, $P < 0.001$).

Table 5. Density of Anopheline Mosquitoes in Houses with Different Wall Types

Wall type	Mos. Density	# Houses	Std. Deviation	95 % CI	
Mat	0.48	532	2.15	–3.734	4.694
Mud	1.10	949	6.17	–10.9932	13.1932
Plaster	0.31	877	1.46	–2.5516	3.1716
Stone	1.21	134	7.40	–13.294	15.714
Thatch	1.66	403	5.17	–8.4732	11.7932
Tin	0.04	24	0.20	–0.352	0.432
Wood	0.02	84	0.15	–0.274	0.314

1.3.6. Indoor Residual Spraying

Indoor residual spraying is applied very selectively to high-risk areas within zones, especially in the NRS, Gash-Barka and Debub. Analysis of the results from the vector distribution survey show that mosquito densities did not differ significantly between sprayed and unsprayed houses ($F_{1, 3018} = 0.048$, $P = 0.827$). This is an important finding in terms of control operations as it brings into question the efficacy of residual

[4] Hudmo house type: Four sided house with roof made of wood and mud; [5] Portable house type: Cone shaped structure made of sticks and covered by mats, and can be moved from place to place.

spraying and therefore the need for careful monitoring of the whole spraying operation. This is especially true in Gash-Barka and NRS zones (Figure 5).

Housing with regard to wall type contributes significantly to the efficacy of the spraying operations. Mosquito densities among sprayed houses of different wall types tended to differ significantly ($F_{6, 643} = 2.403$, P= 0.027). Over 60% (n= 309) of the total *Anopheles* species were collected from sprayed houses with thatch walls. These results show that the level of protection offered by indoor residual house spraying may be compromised by the physical characteristics of the housing. Other forms of control measures are therefore imperative, such as use of insecticide treated bed nets and larval control, under such situations. Table 6 shows the mean densities of mosquitoes collected from sprayed houses with different wall types.

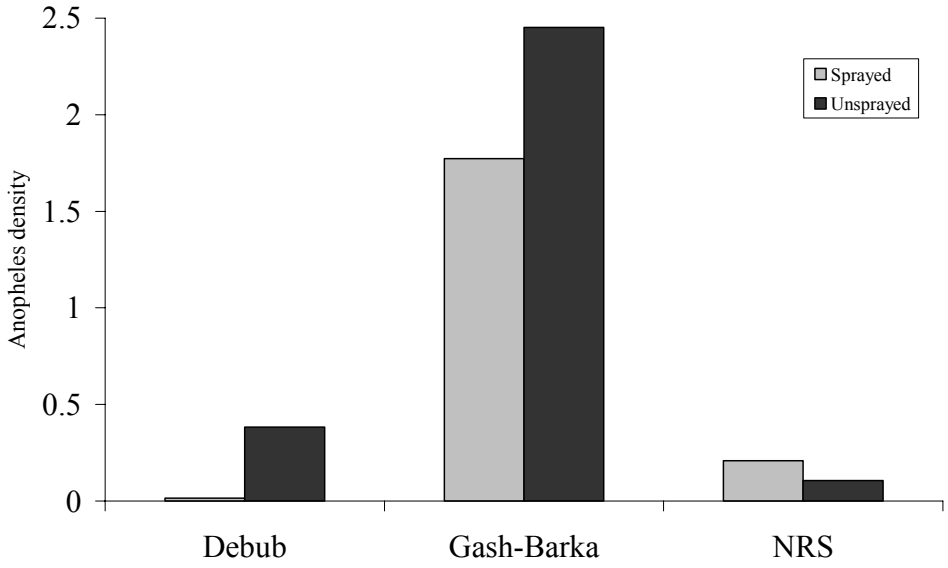


Figure 5. Density of anophelines in sprayed and unsprayed houses

Table 6. Mean Densities of Anophelines in Sprayed Houses with Different Wall Types

Wall type	No. of sprayed houses	Total number of *Anopheles*	*Anopheles* Density	Mean + SD	% of total *Anopheles*
Mat	126	24	0.19	0.19 + 0.62	4.7
Mud	187	173	0.93	0.93 + 4.59	33.7
Plaster	86	1	0.01	0.02 + 0.11	0.2
Stone	24	6	0.25	0.25 + 0.74	1.2
Thatch	208	309	1.49	1.49 + 5.16	60.1
Tin	7	1	0.14	0.14 + 0.38	0.2
Wood	6	0	0	0	0

1.4. Vector Densities and Parasite Prevalence

In 49 villages data were collected on both mosquito densities and malaria prevalence using the OptiMal test[6]. Log transformation [$\log_{10}$ (X + 1)] for both malaria prevalence data and mosquito densities was done to normalize the data. Figure 6 shows the relationship between mosquito densities and malaria prevalence. The results of correlation analysis between mosquito density and parasite prevalence showed a non-significant association between the two variables (Pearson Correlation Coefficient [0.212] was not significant). This result further confirms the fact that mosquito data may not be used alone as a predictive tool for the level of malaria transmission. Other important factors that modulate transmission dynamics have to be considered, such as temperature and humidity, both of which have an influence on the sporogonic cycle and vector longevity. The presence of a gametocyte reservoir in the population and overall vector bionomics also play a critical role. An interaction of all these will therefore determine the vectorial potential of the malaria vector species in question.

The nonlinear relation observed here might be due to the sampling design. Prevalence data were collected on a single occasion in the village, and this may not have captured the time lag between mosquito density and malaria prevalence.

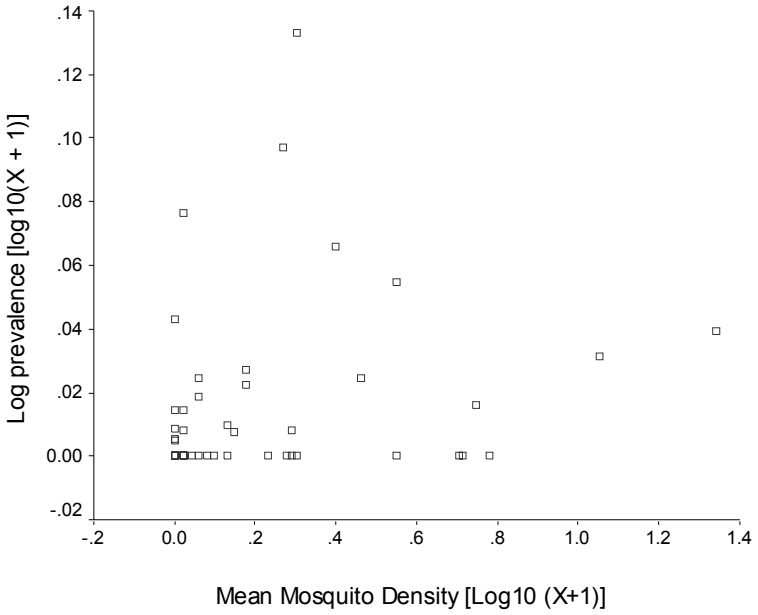


Figure 6. Relationship between mosquito densities and malaria prevalence

[6] OptiMal Test is a Rapid Malaria Diagnostic Test based on the detection of intracellular metabolic enzyme (Parasite Lactase dehydrogenates - PLDH).

1.5. Vector Densities and Associated Ecological Factors

Multivariate techniques were used to analyze the presence of statistical relationships between the environmental variables and *Anopheles* mosquito and larval densities. Logistical regression and linear regression models were run with *Anopheles* densities as dependent variables and with altitude, different Normalized Difference Vegetation Index (NDVI) summaries (Average NDVI, Min NDVI, Max NDVI), rainfall, distance to rivers and latitude and longitude as explanatory variables.

The only variable that showed significant explanation for adult *Anopheles* densities was latitude:, there was a concentration of positive findings of between 14:300 and 16:000º N latitude. Regression models with latitude, longitude, NDVI, altitude and rainfall as predictors could, however, explain only 21.4% of the variation in the *Anopheles* densities. The nonlinear relationship between mosquito densities and the environmental variables can be attributed to the complex nature of interactions between these variables that may have specific time lags that could not be captured by the data. NDVI, longitude and rainfall, on the other hand, showed significant positive correlation with larval densities.

1.6. Conclusions and Implications for Malaria Control

- *An. arabiensis* is the major malaria vector. It may be easy to target vector control measures to such a single vector species than to multiple vectorial systems with variable behavior mechanisms in different ecotypes.

- The vector density indices suggest that human populations in Gash-Barka and Anseba are more predisposed to malaria vector bites; hence there is a greater risk of malaria in these zones. This finding accentuates the need for greater focus in terms of vector control efforts in these two zones. Intrazonal variations observed in the present data, however, show that heterogeneities in risk status have to be addressed even within a given ecological strata.

- Regular monitoring of species densities and composition is needed to continually assess any surges in the size of adult populations of the main vectorial system, *An. arabiensis.* Strengthening of vector surveillance mechanisms through sentinel sites would address this dimension. The choice of sentinel sites should be based on representative ecological strata.

- An evaluation of indoor house spraying technique and the formulation of the DDT/Malathion used is urgently needed. More importantly, the type of housing and the type of wall sprayed may have a strong bearing on the effectiveness of the control method. Therefore other control measures should be offered under situations where intradomicile residual spraying is not workable and bound to be compromised.

- Vector control measures should focus more on the type of housing, thereby saving on effort and cost.

2 Malaria Vector Behavior and Sporozoite Infection Rates

2.1. Seasonal Density of Malaria Vectors

To determine the temporal distribution patterns of *Anopheles* vectors, two villages each were selected in Anseba, Gash-Barka, Debub and NRS zones. In each village ten houses were randomly selected, and indoor resting mosquitoes were sampled once every month by PSC for a period of 24 months. The data presented in this section show the species composition and seasonal variation in mosquito densities and would provide insights into the dynamics of malaria transmission. Knowledge on the population dynamics of malaria vectors is still scanty.

2.1.1. Results

Species Composition and Temporal Patterns

A total of 1,613 anopheline mosquitoes were collected over a period of 24 months. Of these, 75% (n=1213) were collected in Gash-Barka alone. In the rest of the zones, Debub (6.2%), Anseba (17%) and NRS (1.8%), only low numbers of mosquitoes were collected. The results also show that at least 68.4% of the total *Anopheles* mosquitoes collected came from a single village, Hiletsidi in Gash-Barka zone, indicating the heterogeneous nature of vector distribution (Table 7).

Table 7. Variation of Indoor Resting Mosquitoes at the Study Sites

Zone	Study Villages	Sum	N	Mean	% of Total Sum
Anseba	Adi-Bosqual	89	240	0.37 +1.06	5.5
	Hagaz	186	240	0.77 + 4.66	11.5
NRS	Gahtelay	22	200	0.11 + 0.36	1.4
	Ghinda	7	200	0.04 + 0.18	0.4
Gash-Barka	Dasse	107	210	0.51 + 1.47	6.6
	Hiletsidi	1,106	210	5.27 + 10.8	68.4
Debub	Mai-Aini	64	230	0.28 + 1.08	4.0
	Shekaeyamo	36	230	0.16 + 0.55	2.2

At least six *Anopheles* species were collected from the eight study sites. *An. gambiae*[7] made up 97.2% (n=1571) of the total number of indoor resting anopheline mosquitoes

[7] PCR studies indicate that > 99% of positively identified adult and larval specimens are An. arabiensis. This species is therefore the predominant species of the An. gambiae complex in Eritrea.

collected over the 24 months of the study. In all the eight study sites, this species was predominant, indicating its significant position for malaria transmission. The fact that it was collected inside houses adds to its importance as a vector of malaria in the country. The monthly distribution of all anophelines collected in the study is shown in Table 8.

The study has demonstrated the temporal patterns of *An. gambiae,* the principal vector of malaria in the country. In Gash-Barka zone, where over 60% of the total number of species was collected, a bimodal distribution pattern was evident. In January and February, the densities were generally low but rose in March to a density of 5.6 anophelines per household (Figure 7). The density then drops and begins rising again from June to reach the highest peak density (7.4 anophelines/household) in July. It then decreases gradually, though high densities are maintained through November. The distribution observed is coherent with the rainfall pattern at least in the last two quarters of the year. This also could be attributed to changes in humidity and temperature that affect longevity and survival of the species, as well as to the presence of suitable breeding habitats. In the last two quarters of the year, larval breeding goes on in rain pools, ponds, water drainages and on stream edges. The occurrence of high densities in March and April may likely be explained by at least precipitation during this period. However, no ground data on rainfall were collected during the study to validate this supposition. Based on data from the larval ecology survey conducted in the zone, larval breeding activity is maintained in drainage channels at water collection points as well as in stream pools.

In Anseba and Debub, mosquito densities increased from June with peak indoor densities being recorded in September and October, respectively. This occurrence is likely a function of the onset of the rainy season. However, the densities in these two zones are lower as compared to Gash-Barka zone, though larval data indicate that high levels of larval breeding activity are maintained throughout the year. This raises the important question of the number of larvae that develop to reach adult stage in different breeding habitats on a temporal scale. In the NRS zone the low densities of *An. arabiensis* collected did not suffice for any meaningful interpretation.

Table 8. Count of Anopheline Mosquitoes Collected in Eritrea

	Anseba		Debub		Gash-Barka		NRS		
Month	*An. gambiae*	Other species	*An. gambiae*	Other species	*An. gambiae*	Other species	*An. Gambiae*	Other species	Total
Jan	1	2	2	2	9	0	4	0	20
Feb	0	3	0	0	28	0	3	0	34
Mar	0	3	0	5	224	0	2	0	234
Apr	2	1	0	0	153	0	4	0	160
May	0	0	1	0	6	0	0	0	7
Jun	0	1	0	0	4	0	0	0	5
Jul	18	2	5	0	148	0	1	0	174
Aug	19	0	14	0	139	0	0	0	172
Sep	134	0	23	2	257	1	7	0	424
Oct	64	0	31	6	109	0	0	0	210
Nov	17	4	2	5	122	1	5	0	156
Dec	0	2	0	2	12	0	1	0	17
Total	**255**	**18**	**78**	**22**	**1,211**	**2**	**27**	**0**	**1,613**

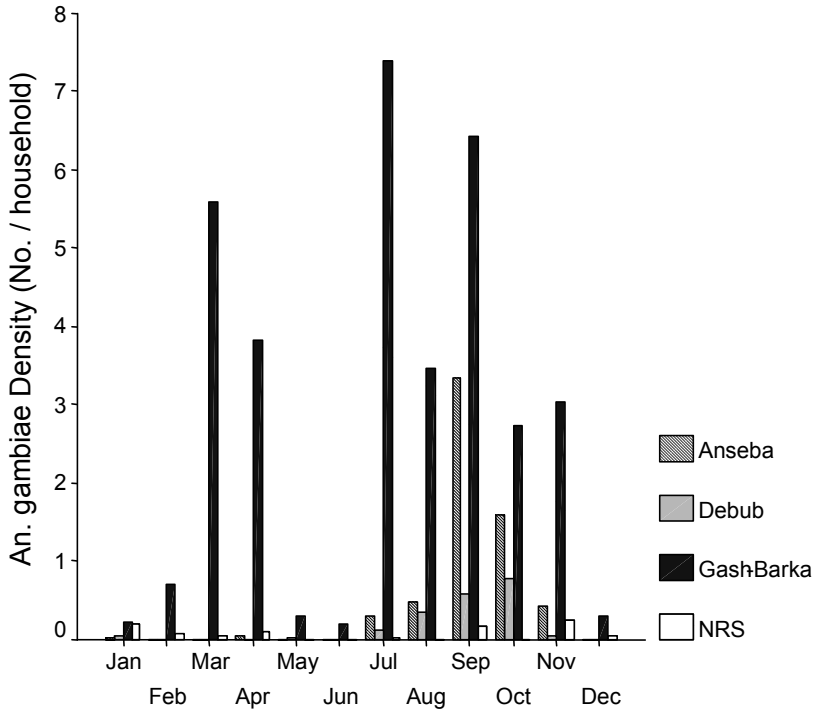


Figure 7. Distribution of *An. gambiae* in Four zones in Eritrea (Oct. 1999–Sept. 2001)

2.1.2. Conclusions and Implications for Control

- *An. gambiae* is the most abundant *Anopheles* species in the sites sampled from the different ecological zones of the country. The species displays distinct temporal patterns that have a perfect fit with the rainfall sequence. Vector control measures, including selective residual spraying, bed net distribution and use, and larval control have to be sustained throughout the year, especially in Gash-Barka zone. Selection of control measures, however, should be guided by intensity of vector densities. In Anseba zone, for example, vector control activities should target the period between July and November, but this should not preclude control activities during the rest of the year.

- High densities of *An. gambiae* were recorded in Gash-Barka and Anseba zones. This reinforces further the need for even greater attention to these two zones with regard to malaria control efforts. Similarly, areas of equally high risk of malaria have to be addressed based on risk maps that have been generated.

- As mosquito breeding and increase in mosquito population densities are influenced by rainfall, temperature and humidity, an assessment of the correlation between these climatic factors and vector densities should be investigated. Information generated could be used for predicting the onset of increased vector densities under similar ecologies. Predicting the onset of the rains and the occurrence of peak mosquito densities are important for the timing of control activities.

2.2. Biting Behavior of Malaria Vector Species

Studies were carried out at two houses in each of the two vector behavior study sites in each zone. Sampling of mosquitoes was conducted by human landing catches from 6:00 p.m. to 6:00 a.m. once per month for 24 months in each site. The collectors worked in pairs, one pair working from 6:00 p.m. to midnight and the next from midnight until 6:00 a.m. A pair of collectors worked indoors, and a second pair outside at a distance of about 20 m away from the house. The teams rotated through the sentinel houses on different nights. Mosquitoes collected were stored in paper cups and processed separately. Data on relative humidity and temperature were recorded.

2.2.1. Results

Vector Abundance and Biting Cycle

A total of 2,711 anopheline mosquitoes were collected on the human baits over the 24-month study period. *An. gambiae* comprised of 97.6% (n= 2645) of the total mosquitoes collected. Of the total mosquitoes collected 43.3% (n=1174) were sampled indoors, and 56.7% (n= 1537) were collected outdoors. The difference between indoor and outdoor biting densities was significantly different ($t = -4.307$, $df= 4139$, $P < 0.001$).

Comparison of biting activity for *An. gambiae* between zones revealed significant differences in mosquito numbers sampled both indoors ($F_{3, 4139}$= 36.017, P< 0.001) and outdoors ($F_{3, 4139}$= 32.819, P< 0.001). This indicates further that the risk of infection as predicted from vector abundance is highly variable. Therefore, the need is great for vector control efforts to be targeted to hot spots.

The time for peak biting activity for the anopheline species was variable between the sites. In the Gash-Barka zone mosquito biting commenced at 6:00 p.m. but steadily increased with a peak biting activity being between 9:00 p.m. and 11:00 p.m. outdoors and from 1:00 a.m.–2:00 a.m. indoors. Though a general decrease in landing collections was observed from 11:00 p.m.–2:00 a.m. both outdoors and indoors, respectively, appreciable levels of biting activity was maintained throughout the night.

In the sites studied in Anseba zone, biting activity by *An. gambiae* began at 6:00 p.m. with peak activity outdoors being observed between 7:00 p.m. and 11:00 p.m. Indoor-biting activity started at 6:00 p.m. An extended period of activity continued from 7:00 p.m. and peaked between midnight and 3:00 a.m. The pattern observed in Anseba and Gash-Barka zones are evidently similar, suggesting that the behavior mechanisms of this species are not altered to a great extent by altitude and other ecological factors.

In Debub zone, sustained high levels of biting activity by *An. gambiae* indoors and outdoors were concentrated between 8:00 p.m. and 3:00 a.m. This species showed a similar pattern as in Gash-Barka and Anseba zone. Figure 8 shows the biting cycle of *An. gambiae* expressed as densities collected per hour in these three zones. Data from the NRS were insufficient for any meaningful interpretation.

Data collected on the other anopheline species (2.4%, n= 66) over the 24 months of study could not provide any meaningful interpretation. However, of the total collected, 63.6% (n= 42) were sampled in outdoor human landing collections.

Figure 8. Biting cycle of *Anopheles gambiae* in Anseba, Gash-Barka and Debub zones

Analysis of the data based on altitudinal categories revealed similar tendencies of anopheline biting rhythms as observed in individual zones. This could be attributed again to the fact that the study deals with only one species, *An. gambiae,* whose behavior patterns remain stable or unaltered under the different ecological situations. Biting generally commences at 6:00 p.m. and peaks up between 7:00 p.m. and 11:00 p.m. There is significant biting activity indoors and outdoors throughout the night (Figure 9).

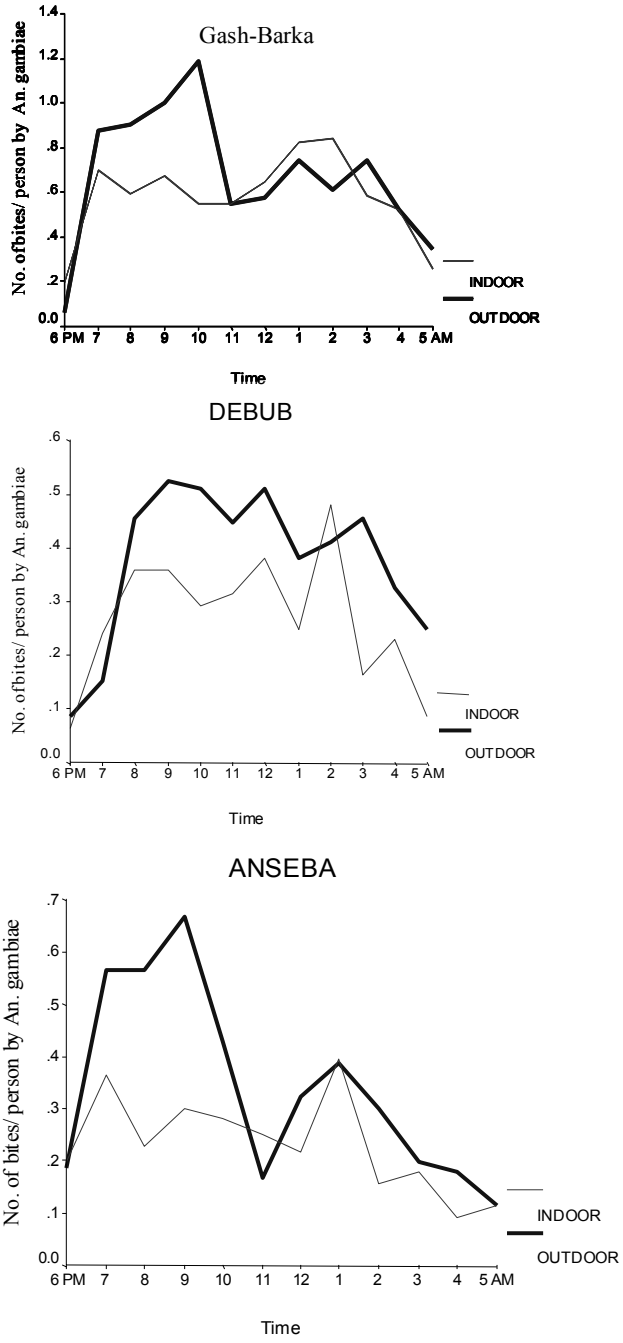


Figure 8. Biting cycle of *Anopheles gambiae s.l.* in Anseba, Gash-Barka and Debub zones

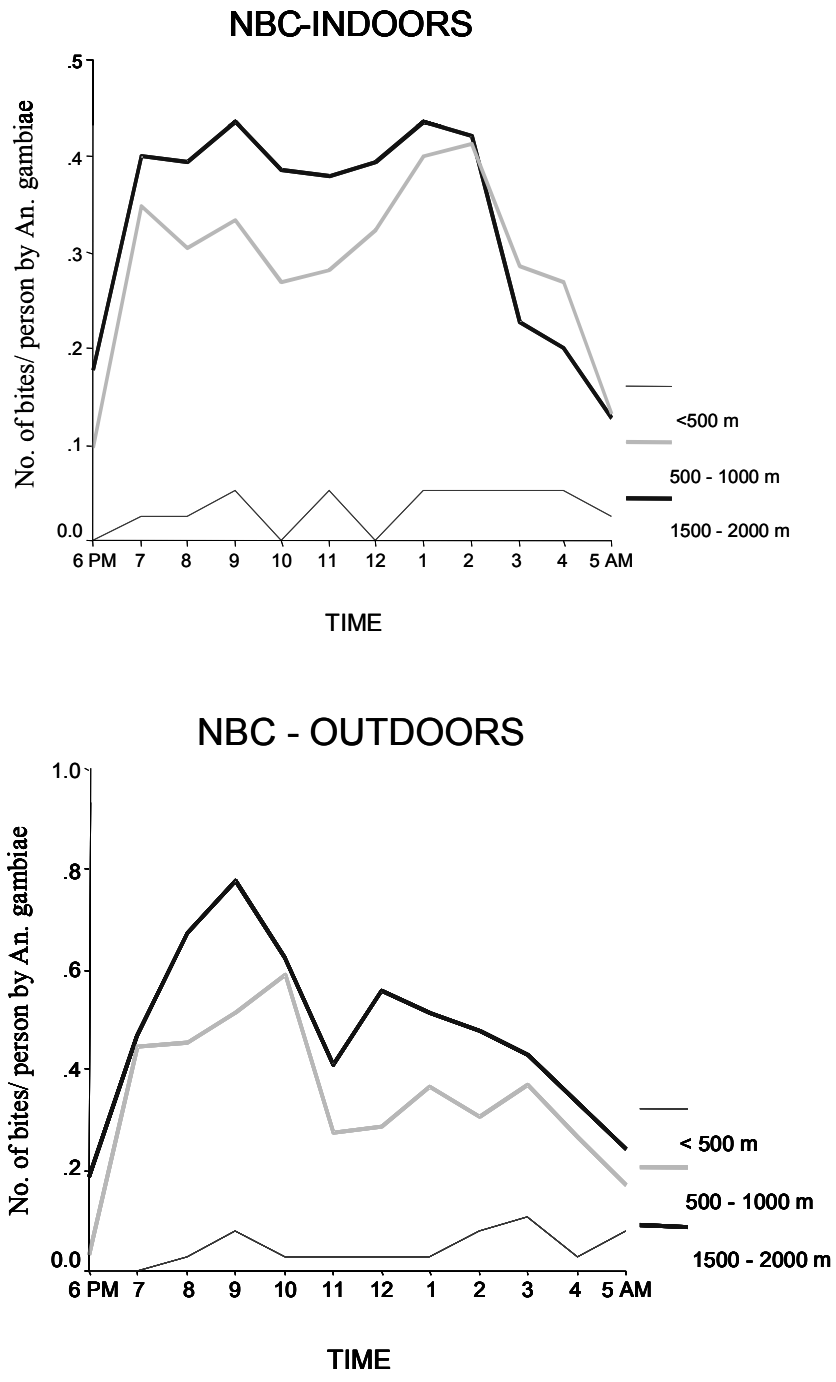


Figure 9. Biting cycles for *Anopheles gambiae* in different altitudinal zones

2.2.2. Conclusion and Implications for Control

- The fact that there is a high tendency for the malaria vectors to bite outdoors provides an important challenge for control. There is a need to reassess the role of bed nets in light of these findings with a view of meeting the challenge by integrating other measures into malaria control. This would include investment of

time and other resources to improve control of pre-adult stages. Dissemination of information and sensitization of the population through mass media on the importance of personal protection is a critical action for consideration.

- The biting rhythm for *An. gambiae* between sites was evidently similar, suggesting that some behavioral traits are conserved despite the large ecological variation between the sites sampled. However, this may suggest active exchange of genetic material across the geographical barriers, an area that would need further study to establish any genotypic variation in *An. gambiae* populations across ecological strata.

2.3. Resting Behavior of Malaria Vectors

Within each sentinel village two pits serving as outdoor mosquito shelters were constructed. One pit was located at the periphery of the village and the other at the center. Mosquito collections were conducted monthly for three consecutive days by use of aspirators. The mosquitoes collected were preserved in petri dishes lined with moist cotton and later identified to species. At every site ten sentinel houses were sampled by PSC to estimate the densities and composition of indoor resting anopheline mosquitoes.

2.3.1. Results

A total of 1,359 anophelines were collected from outdoor resting shelters or pit shelters. *An. gambiae* was the predominant species collected from the pit shelters and comprised of 87.3% (n= 1186) of the total anophelines collected. Other species present in low numbers included *An. cinereus* (10.4%), *An. pretoriensis* (0.3%), *An. d'thali* (0.9%), *An. squamosus* (0.2%), *An. demeilloni* (0.8%), *An. garnhami* (0.1%) and *An. rupicolus* (0.1%).

Over the same period some 1,613 endophilic[8] anopheline mosquitoes were sampled from ten randomly selected houses per village. *An. gambiae* made up 97.2% of the total anophelines collected. The relative proportions of anophelines resting indoors and outdoors are shown in Table 9. The results show distinct variation in resting behavior of *An. gambiae* in the different zones (Figure 10). In the sites sampled in Debub zone (altitude above 1,500 m) *An. gambiae* had greater exophilic tendencies. A similar trend was also observed in NRS for the same species. In Anseba zone, the number of indoor and outdoor resting species were generally similar. In Gash-Barka a high proportion of *An. gambiae* mosquitoes rested indoors. This distinct variation in behavior pattern may suggest the existence of different subpopulations of *An. gambiae* species in different ecological zones of the country. DNA based molecular assays would need to be conducted in order to establish any genetic variation between the different *An. gambiae* populations. It is possible that the shift in resting tendencies

[8] Endophily: Tendency to rest indoors

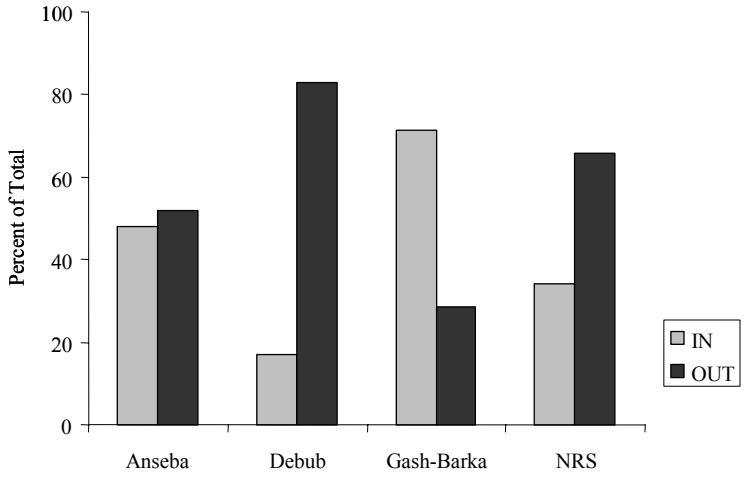
observed in this species results from the intensity of indoor residual spraying in the different regions.

Table 9. Proportion of Mosquitoes Resting Indoors and Outdoors in Anseba, Debub, Gash-Barka and NRS Zones

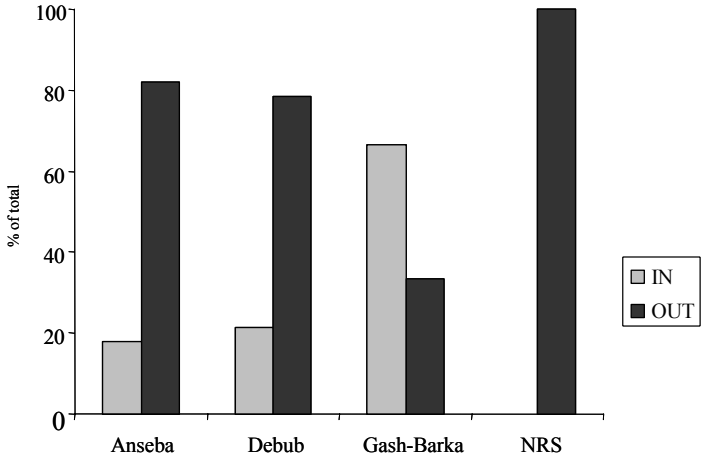
	An. Gambiae		Other *Anopheles* species	
Zone	**IN**	**OUT**	**IN**	**OUT**
Anseba	255 (48%)	276 (52%)	18 (18%)	82 (82%)
Debub	78 (17%)	380 (83%)	22 (22%)	80 (78%)
Gash-Barka	1,211 (71%)	487 (29%)	2 (67%)	1 (33%)
NRS	27 (34%)	52 (66%)	0 (0%)	10 (100%)
Total	**1,571**	**1,195**	**42**	**173**

*Values in parenthesis represent the proportion of anopheline species

A. An. Gambiae s.l.



B. Other *Anopheles* species



A. *An. gambiae*

Figure 10. Proportion of endophilic and exophilic mosquitoes

2.3.2. Conclusions and Implications for Control

The results indicate that vector behavior needs to be considered in the implementation of control activities. Indoor residual spraying, effective mainly against endophilic species, would be a reasonable option in Gash-Barka zone, where over 70% of the major vectorial system is endophilic. In the other zones its efficiency would be compromised by the high tendencies of exophily observed, and an integrated approach to vector control would be more suitable.

An integrated approach to vector control, involving use of bed nets and larval control would be a productive approach in the Debub, NRS and Anseba zones.

2.4. *Plasmodium falciparum* Sporozoite Rate Determination

In order to measure the infectivity of anopheline mosquitoes and to assess the vectorial status of the different anopheline mosquitoes collected in both the vector distribution and behavior study, mosquito specimens were analyzed using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. Briefly, the head and thorax were separated from the rest of the body and assayed for presence of the circumsporozoite protein antigen in the salivary glands using monoclonal antibodies specific for *Plasmodium falciparum*. A total of 6,634 anopheline species were assayed.

2.4.1. Results

Variation in *P. falciparum* Sporozoite Rates by Zone

Overall, 0.99% (n=66) infection rate was recorded from the total number of mosquitoes tested (n= 6,634). This value is generally low compared to figures recorded from other malarious regions in sub-Saharan Africa. However, this does not preclude the fact that such low infection rates would be responsible for sufficiently high levels of malaria in the population. Partitioning the infection rates by zone indicated a 1.3% infection rate in Gash-Barka, which is the most highly malarious zone in the country. The infection rates recorded in Anseba and Debub zones were 0.5% and 1.01%, respectively. In Maekel zone, one anopheline mosquito collected at an altitude greater than 2,000 m was found to be positive, giving a sporozoite rate of 1.3% (Table 10). However, it should be noted that the number of mosquitoes collected and subsequently tested from this zone was very low (n= 80). No infected mosquitoes were observed from specimens collected from the NRS zone. The difference in number of infected mosquitoes between zones was not significantly different (χ^2=9.923, df= 4, P= 0.42).

Analysis of the data at village level showed that at least 65.1% (n= 44) of the positive mosquitoes were collected from two sites in Gash-Barka zone. Hiletsidi (altitude 570 m) alone had 59.1% (n= 39) of the total positive mosquitoes. This observation shows the variable nature of the risk of malaria infection and also the fact that risk is site specific. It underlines the importance of generating intensive information on risk status across ecological strata.

Of the total number of specimens that tested positive for *P. falciparum* sporozoite antigen, 96.7% (n=64) were *An. gambiae.* Two other species, *An. d'thali* and *An. cinereus,* were positive. This result further reveals the important status of *An. gambiae* in transmission of malaria in the country. Studies to assess the population dynamics and biology of *An. d'thali* and *An. cinereus* would be desirable, as these two species may be critical to the transmission of malaria under appropriate environmental conditions.

These results of *Anopheles* infection rates are useful in estimating the risk of malaria in different zones of the country. However, the entomological inoculation rate (EIR), calculated as a product of the man biting rate and the sporozoite rate, could not be derived from these data because of low figures of sporozoite rates.

Table 10. *P. falciparum* Infection Rates in *Anopheles gambiae* and Other Anopheline Species

Zone	# Tested	# Positive	Sporozoite Rate (%)
Anseba	1,849	9	0.49
Debub	1,185	12	1.01
Gash-Barka	3,358	44	1.31
Maekel	80	1	1.25
NRS	162	0	0

The number of infected anopheline mosquitoes varied significantly with the method of collection, i.e., PSC, pit shelter collection and human landing catches indoors and outdoors (χ^2=15.59, df= 3, P= 0.01). Predominantly high numbers of positive mosquitoes were collected indoors by PSC. The results further show that appreciable biting goes on outdoors by infected mosquitoes (Table 11).

Table 11. Proportion of Positive Anophelines Collected Using Different Techniques

Collection Technique	# Tested	# Positive	% of Total Positive
NBC-Indoors	1,001	21	31.8
NBC-Outdoors	1,567	12	18.2
Pit shelters	1,129	12	18.2
PSC	2,937	21	31.8

Temporal Variation in *P. falciparum* sporozoite Rates in Hiletsidi

Analysis of the data further showed that there was significant variation in *An. gambiae* on a temporal scale in Hiletsidi, Gash-Barka zone (χ^2=90.11, df= 22, P< 0.001). Generally low levels of infection were recorded between November and February. The highest proportion of infected mosquitoes was collected in September, though infection rates tended to rise between July and October, which coincides with the rainy season in this zone (Figure 11). This could therefore be attributed to favorable conditions such as increased humidity and optimal temperatures that would affect mosquito survival and development of parasites in the mosquito. High densities of anopheline species also were present following the rains that occur between July and October. The presence of infected mosquitoes between December and May, a period presumed to be free of malaria, clearly points to the need for sustained vector control operations year round.

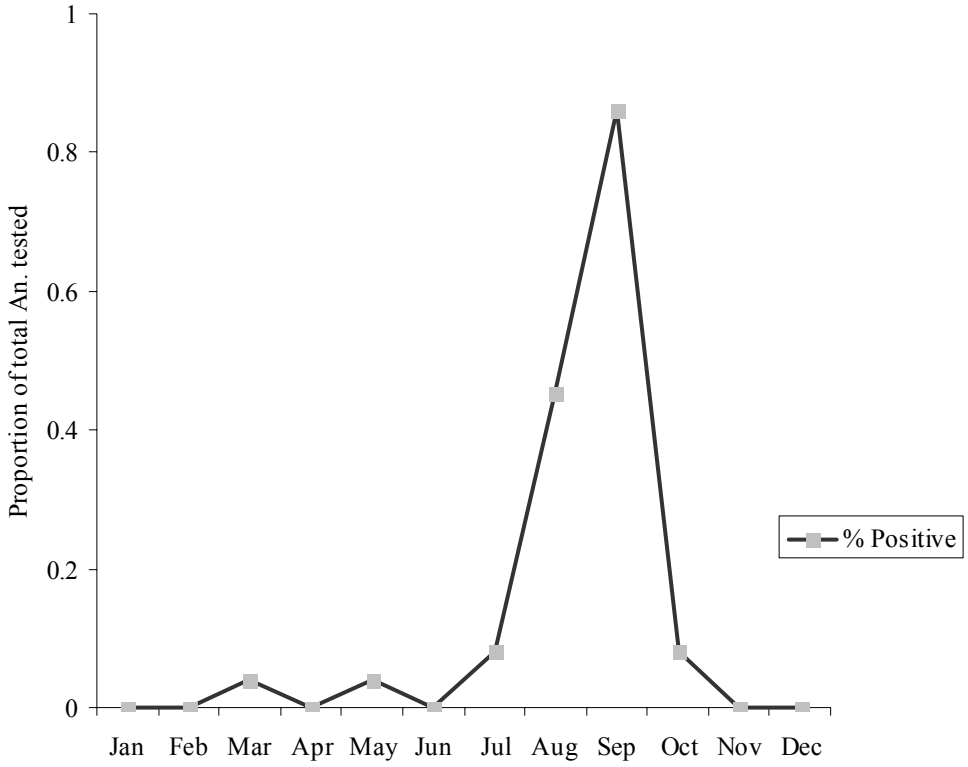


Figure 11. Temporal pattern of infection rates in *An. gambiae* in Hiletsidi, Gash-Barka zone

Variation in *P. falciparum* Sporozoite Rates by Ecological Strata

Eritrea can be divided into five distinct ecological strata based on altitude, rainfall and vegetation. These include:

- Highlands above 2000 m: Highlands of Debub and Maekel with moderate rainfall in the main rain season.
- Low wet western plains: Areas lying between 500 m and 1,000 m in the southwestern portion of the country with annual rainfall levels above 400 mm per year.
- Western escarpments and valleys: Areas between 1,000 m and 2,000 m with annual rainfall above 500 mm.
- Eastern escarpments: Areas lying between 200 m and 500 m, and 2000 m with rainfall above 200 mm. Malaria is focal and related to proximity to breeding sites; prevalence may be high in such areas such as Ghinda.
- Dry lowlands: This includes the arid and the semiarid zones from the national map with rainfall < 200 mm (NDVI < 92) and altitudes below 500 m.

Analysis of sporozoite data reveals that infected mosquitoes were found in only two ecological zones (Table 12). At least 66.7% (n= 44) of the total infected mosquitoes were derived from the low wet area, which comprises the bulk of Gash-Barka zone. These areas receive over 400 mm of rainfall annually, making breeding and survival of adult mosquitoes possible. The rest of the infected mosquitoes (33.3%, n= 22) were sampled from the western escarpments and valleys lying between 500 m and 2,000 m, with rainfall amounts totaling over 500 mm per annum. The data present a clear picture of risk based on infectivity rates in mosquitoes. In the other ecological zones malaria is generally focalized with high prevalence being associated only with proximity to breeding sites.

Table 12. Comparison of *Plasmodium falciparum* Infection Rates in *Anopheles* Mosquitoes in Different Ecological Zones

Ecological zone	# Tested	# Positive	Sporozoite Rate (%)
Southwestern low wet zones, 500–1000 m, >400 mm rainfall per annum	3,358	44	1.31
Western escarpments, 1000–2000 m, > 200 mm rainfall/ annum	3,035	21	0.69
Highlands above 2000 m	79	1	0
Eastern escarpments, 200–500 m and 2000 m, rainfall > 200 mm per annum	57	0	0
Dry lowlands, <500 m, rainfall < 200 mm (NDVI < 92)	105	0	0

2.5. Feeding Behavior of the Anopheline Mosquitoes in Eritrea

The vector status of an anopheline mosquito may be expressed by its feeding tendency on humans. Mosquitoes that are known to feed exclusively on nonhuman hosts are usually poor vectors. A total of 2,820 fully fed and half-gravid mosquito specimens were tested by the ELISA test to determine blood-feeding preferences of the *Anopheles* species. The specimens were tested using antibody conjugates specific for human and bovine antigens.

2.5.1. Results

Feeding Preferences

The results indicate that at least 54.9% of the total number of anophelines received blood from human sources. A fairly large proportion (34.5%) had a mixed blood meal of human and bovine blood while 12% derived their blood meal only from a bovine source (Table 13). Nearly all the species tested showed fairly high preferences for human blood except for *An. cinereus* and to some degree *An. d'thali,* which fed equally on bovine hosts. Nonetheless, the fact that at least a proportion of *An. d'thali* and *An. cinereus* were found to be positive for *P. falciparum* sporozoites may reflect

the importance of these species for the transmission of malaria if conditions become favorable. These two species are restricted to high altitude areas. Climatic changes that are occurring globally may impact the vectorial status of these potential vectors. Regular monitoring of species composition and vector densities will become necessary for an effective malaria control program.

The human blood index recorded for *An. gambiae* of 0.5 is generally low as compared to studies conducted elsewhere and this may have an impact on malaria transmission. The EIR, which expresses the risk of malaria transmission as a function of sporozoite rate, biting rate and the human blood index, is generally low when frequency of feeding on human host is low. Data from PCR analysis of both *An. gambiae* larvae and adults indicate that over 99% is *An. arabiensis.* Compared to *An. gambiae s.s., An. arabiensis* shows less tendency to feed on human hosts (anthropophily). This tendency may reduce its vectorial significance relative to *An. gambiae s.s* where both species occur. A total of 854 anophelines assayed using antibody conjugates specific to human and bovine antigens did not produce a positive signal. Of these, over 80% were *An. gambiae.* Further analysis of the negative samples for other hosts such as goat, sheep, donkeys and horses that are present in most of the villages surveyed would be necessary to get a full picture of feeding range of the vector species.

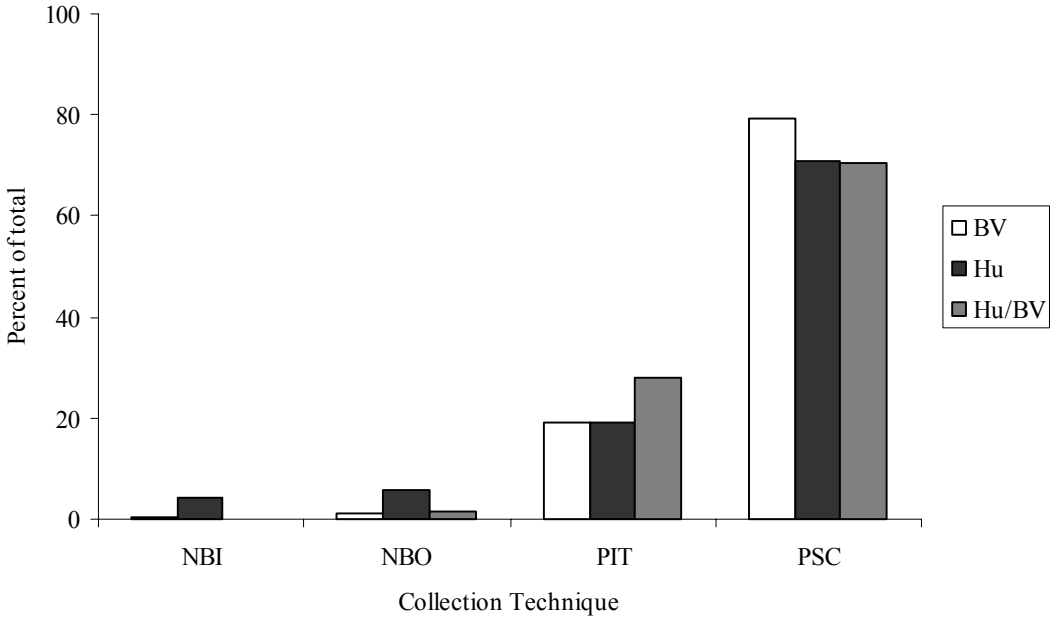
Table 13. Blood Meal Sources of *Anopheles* Species Collected in the Distribution and Vector Behavior Studies

	Blood Meal sources				
Anopheles **species**	**Human**	**Bovine**	**Human /Bovine**	**Negative for Human & Bovine**	**Total Tested**
An. gambiae	990	509	260	715	2,474
An. cinereus	39	45	19	52	155
An. chrysti	2	0	0	0	2
An. d'thali	29	11	21	65	126
An. demeilloni	2	2	0	1	5
An. funestus	1	1	0	0	2
An. garnhami	3	1	0	0	4
An. pharoensis	2	0	0	0	2
An. pretoriensis	4	1	0	6	11
An. rhodesiensis	0	0	0	1	1
An. rupiculos	5	1	4	7	17
An. squamosus	12	2	0	7	21
Total	1,089	573	304	854	2,820

Blood meal source and collection technique

The results suggest that the highest proportion (70.8%) of anopheline species positive for the human blood meal test were collected indoors. However, it is notable that at

least 20% were collected resting outdoors in pit shelters, suggesting some tendency of exophily, considering that these species could have obtained their blood meal indoors (Figure 12). The fact that both infected mosquitoes and human blood fed mosquitoes rest outdoors poses a challenge to vector control operations that target indoor resting species. An integrated approach to vector control is the only viable solution.



NBI = Night biting collection – Indoors; NBO = Night biting collection – Outdoors
PIT = Pit shelter collection; PSC = Pyrethrum spray collection

Figure 12. Variation in anopheline blood meal sources in different collection techniques

2.5.2. Conclusions and Implications for Control

- The human blood index for *An. gambiae* (0.5) is low and this could be a contributing factor to the level of malaria transmission. Nonhuman hosts present in kraals close to human dwellings could be acting as a barrier. This species show high tendencies of feeding outdoors and mainly on nonhuman hosts.

- The exophily observed indirectly through human blood-fed species in outdoor pit shelters may compromise efficiency of intradomicile control targeted to this species. This calls for incorporation of other measures such as effective larval control. Periodic monitoring of vector behavioral tendencies is recommended in order to assess any shift that would predispose the population to greater risk of infection. Use of insecticides is known to accelerate building of avoidance mechanisms to sprayed structures.

3 Larval Ecology of Malaria Vectors

3.1. Introduction and Rationale

Malaria transmission is dependent on the presence of efficient vectors. These arise from suitable breeding habitats of the anopheline species that contribute to the transmission of malaria. Understanding of larval dynamics in a malaria setting remains critical if efficient control of malaria vectors is to be achieved. For larval control to be an integral part of a vector management program, a sound understanding of the factors responsible for breeding activity of the principal vectors of malaria is necessary. In Eritrea, *Anopheles arabiensis* (Diptera: Culicidae) is considered to be the major malaria vector. However, information on the dynamics of the pre-adult stages of this species and other important anopheline species is very scanty. A strong association exists between distribution of the pre-adult stages and that of the adult vectors. Knowledge of the influence of habitat factors on larval production would be critical for understanding the spatial and temporal distribution patterns of the anopheline species. The present study was conducted to determine the spatial and temporal distribution of anopheline species in relation to habitat diversity.

3.2. Spatial and Temporal Larval Distribution Studies

Larval surveys were conducted at each of the 305 villages sampled for adult mosquitoes in the vector distribution survey. All breeding sites present in and around the village were sampled for anopheline larvae using standard dipping techniques. At two sites designated for longitudinal studies of malaria vectors in Anseba, Debub, Gash-Barka and NRS zone, larval habitats were sampled once a month for 24 months for anopheline larvae. The aim of the study was to establish the habitat types, vector composition and temporal variation in productivity. The types of breeding habitats, number of anopheline larvae and number of dips were recorded. All third and fourth instar anopheline larvae were then preserved in absolute alcohol and later identified to species. A sample of *An. gambiae* larvae was analyzed by PCR assays to determine the sibling species composition of the complex. The physical characteristics of the breeding habitat were also recorded. These included water current, depth, presence of vegetation, amount of shade, water turbidity and water temperature.

3.3. Results

3.3.1. Larval Abundance

Anopheline larvae were sampled predominantly from edges of streams and water puddles on drying riverbeds, rain pools, ponds, dams, swamps and drainage channels at communal water supply points. The mean density of *Anopheles* larvae over the two

sampling efforts was 39.41 larvae per 100 dips. There was variable contribution of each of the breeding habitats with regard to larval production (Table 14). Significantly higher densities of larvae were sampled from streams and rivers ($F_{7,261}$ = 4.395, P< 0.001). Figure 13 illustrates the relative importance of streams and rivers, rain pools and ponds as important breeding sites for malaria vectors in the country. The other breeding sites, though less productive, may potentially be important under appropriate environmental conditions. Streams and rivers were the most productive breeding sites for the anopheline species forming over 90% of the total anopheline larvae sampled. Of the total number of breeding sites sampled, over 60% (n= 163) were stream or rivers, an indication that they are of greater significance with regard to mosquito production and malaria transmission in most parts of the country. Diversity of breeding sites was also evident at zonal level from this survey. In Gash-Barka zone alone, larval breeding was found in at least four different habitats: streams and rivers, rain pools, swamps and run off channels at communal water supply points (Figure 14). This is a reflection of the need for accurate mapping of all breeding sites in this area and subsequent cataloging of productivity on a temporal scale in order to establish the temporal significance of each type of breeding site. Most of the rivers are temporary and breeding goes on only at specified times of the year, which coincides with the peak malaria transmission season. In zones and subzones where breeding takes place mainly on the temporary streams, control efforts should be targeted at these breeding sites throughout the year. Larval control pilot studies currently being undertaken would address this dimension with a view of validating the role of larval control on malaria transmission. Table 15 shows the distribution of the various species identified among the different breeding sites. These data show the diversity and significance of different breeding habitats for malaria vectors, which is an important step in planning larval control interventions.

Table 14. Density of Anopheline Larvae Collected from Different Types of Breeding Sites in Anseba, Debub, Gash-Barka, Maekel, NRS and SRS Zones

Breeding habitat	Number of breeding habitats	No. of *Anopheles* larvae	Density (no./100 dips)	Percent of total
Barrels	2	0	0.00	0
Dams	18	16	0.36	0.2
Ponds	35	316	20.89	3.0
Rain pools	15	333	20.64	3.2
Streams/Rivers	163	9,481	55.42	91.2
Swamps	12	155	12.85	1.5
Water supply/Wells	12	90	7.50	0.9
Wells	5	0	0.00	0

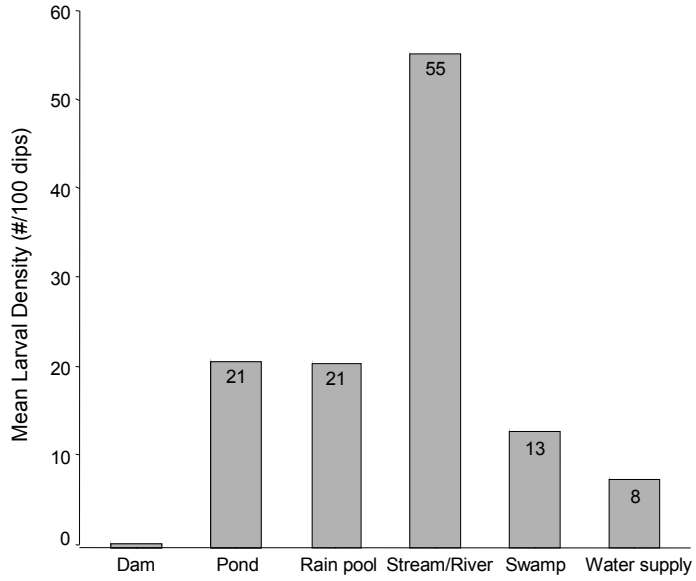


Figure 13. Densities of anopheline larvae in different breeding habitats

3.3.2. Larval Composition

At least 50% of the total anopheline larvae collected were identified to species by morphological criteria. A total of eight anopheline species were identified. *An. gambiae* and *An. cinereus* were the predominant species while other species were represented only in low proportions (Table 15). An important observation from this survey is that the main vector species, *An. gambiae,* was found to breed in at least five habitat types in the different ecological zones, showing the versatility of this species and therefore its important vectorial status in malaria transmission. Further analysis to determine the factors responsible for the variation in larval densities will be necessary.

Table 15. Species Distribution of Anopheline Larvae in Different Breeding Habitats

Species	Dam	Pond	Rain-Pools	Stream/ Rivers	Swamp	Water Supply
An. funestus				✓		
An. pretoriensis	✓			✓		
An. squamosus				✓		
An. adenensis		✓		✓		
An. cinereus				✓		
An. demeilloni				✓		
An. d'thali				✓		
An. gambiae		✓	✓	✓	✓	✓

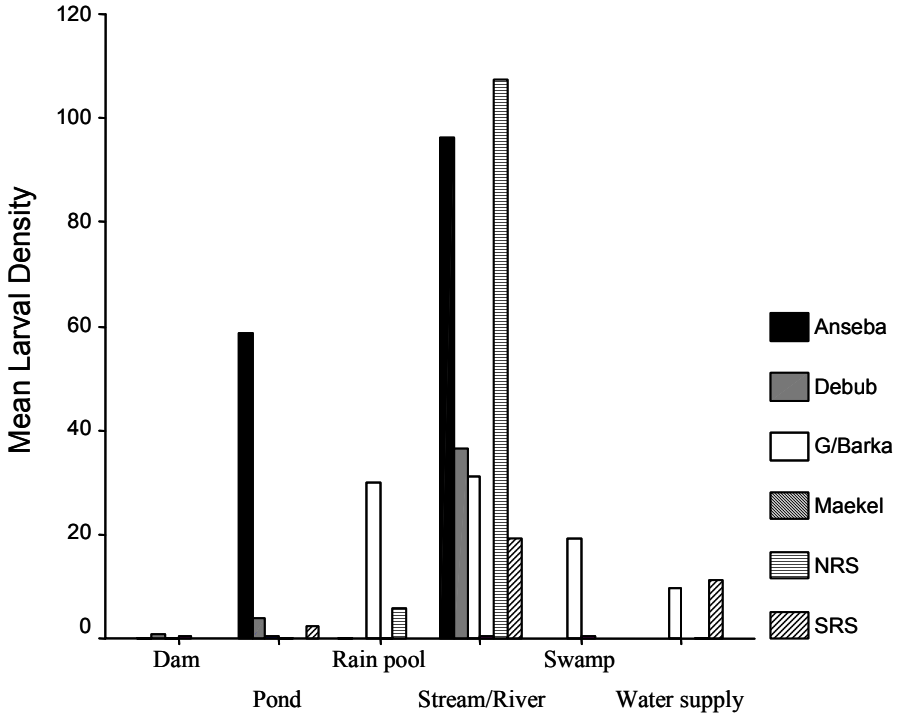


Figure 14. Variation in anopheline larval densities in different breeding habitats by zone

3.3.3. Factors Associated with Larval Breeding in Different Habitats

Data were collected on a range of factors that would affect the production of larvae and therefore explain variability in densities of larvae. These included presence of vegetation (floating or emergent), habitat depth, water turbidity, water current, permanence of breeding site, amount of shade and water temperature. Significantly higher densities of anopheline larvae were collected in breeding sites that were clear, shallow and slow moving (Table 16). Presence of emergent or floating vegetation, intensity of shade, and permanence of breeding site did not seem to significantly affect larval density.

Table 16. Characteristics of breeding sites and mean densities of *Anopheles* larvae

Habitat characteristics		Mean + SD	F	Sig.
Intensity of shade	Light	38.9 + 64.9	0.258	0.612
	Shade	47.7 + 63.5		
Turbidity	Clear	42.6 + 67.9	4.093	0.044
	Turbid	19.3 + 32.3		
Water depth	Deep	6.25 + 12.1	13.79	0.000
	Shallow	45.7 + 68.6		
Vegetation	Emergent	25.8 + 41.5	1.507	0.213
	Floating	50.5 + 79.3		
	Emergent + Floating	37.5 + 37.6		
	None	39.8 + 65.6		
Permanence	Permanent	39.9 + 75.3	0.031	0.860
	Temporary	38.6 + 45.1		
Water current	Moving	54.9 + 56.2	12.95	0.000
	Still	26.7 + 68.5		

Larval density was positively correlated to change in water temperature (Pearson correlation coefficient, r = 0.173, P = 0.03). Though some variation in larval densities can be explained by habitat factors such as water turbidity, depth, current and temperature, it was evident that interaction between these factors was not significant. This indicates that larval production is a function of complex interaction of habitat characteristics, some of which were not measured in the present survey. In order to gain a better understanding of variability in larval production, measurement of water chemistry on a temporal scale would be necessary. The present study, however, illustrates clearly the importance of some of the physical characteristics of water habitats that play a role in determining the level of production of immature stages of *Anopheles* species. The study shows that that most of the breeding habitats include slow moving rivers; this could be managed through community involvement.

3.3.4. Spatial Distribution of *Anopheles* Larvae

Geographical reference points were taken for each breeding habitat sampled in the vector distribution survey. These values were used to map densities of larvae for all the breeding sites sampled. Figure 15 shows the spatial distribution of larval densities in the country over the two sampling phases (Year 1 and 2). Significantly higher densities of *Anopheles* larvae were sampled during the second phase of study and this may be attributed to a general shift in environmental conditions that could have resulted in higher rainfall amounts. This would in turn lead to production of more breeding sites. The study design did not capture changes in environmental variables over time so as to ascertain the factors responsible for such variability. Collection of weather variables built into the pilot studies and subsequent monitoring at sentinel

sites would answer these questions, as well as providing information that could be used to accurately predict changes in larval production.

Larval densities were generally high in the high altitude zones and on the western escarpments of the country lying to the west of longitude 40ºE. These areas lie between 1,000 and 2,000 m above sea level and receive over 500 mm of rainfall annually. Larval abundance was however lower in the western lowlands, which includes most of the Gash-Barka, zone where rainfall amounts are greater than 400 mm. This occurrence could be attributed a number of factors, including proximity of breeding sites to the villages sampled and therefore ease of access by the field teams. Very low densities of larvae were sampled from areas on the coastal strip, which receives generally very low rainfall. However, focal areas of high larval abundance were evident in this zone, indicating that control measures targeted at such limited sites could be effective for the control of malaria through larval control.

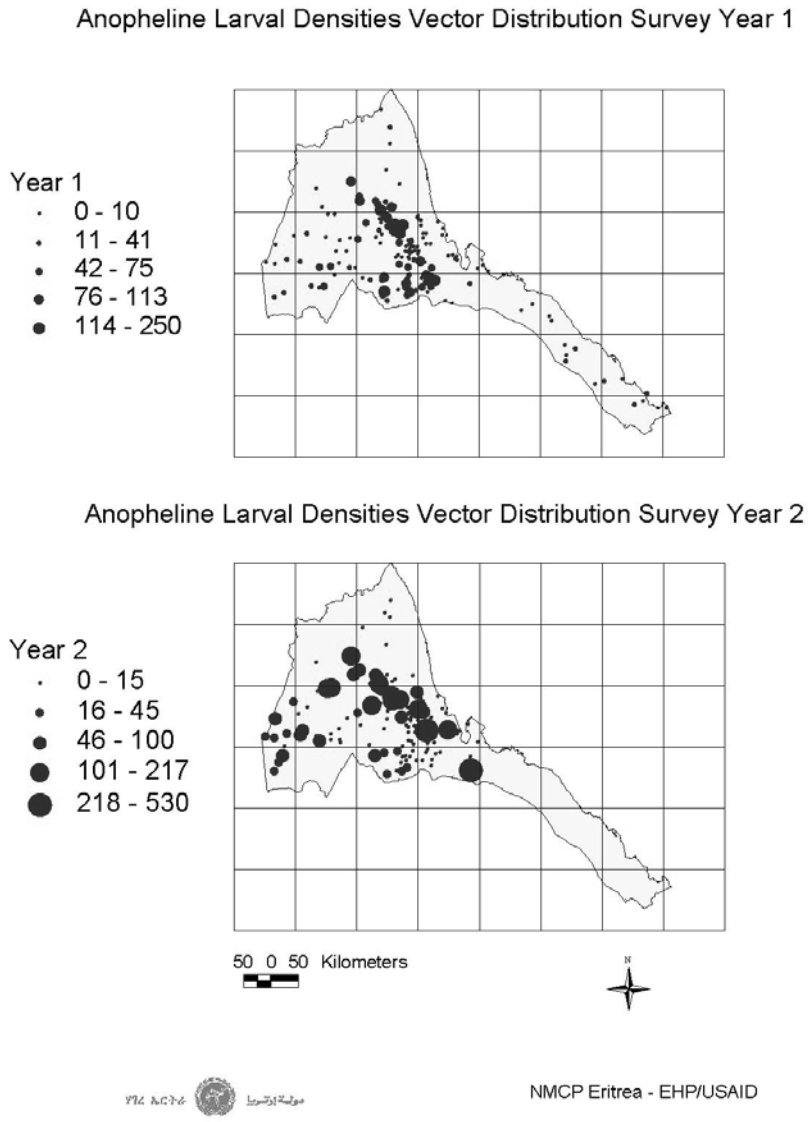


Figure 15. Map of Eritrea showing spatial distribution of *Anopheles* larvae

Correlation analysis of the association between log transformed larval and endophilic adult *Anopheles* densities was positively associated (Pearson correlation coefficient, r = 0.317, P = 0.00). This suggests that the densities of adult mosquitoes may be used to accurately assess the impact of a larval control operation. Figure 16 shows the relationship between larval and adult densities.

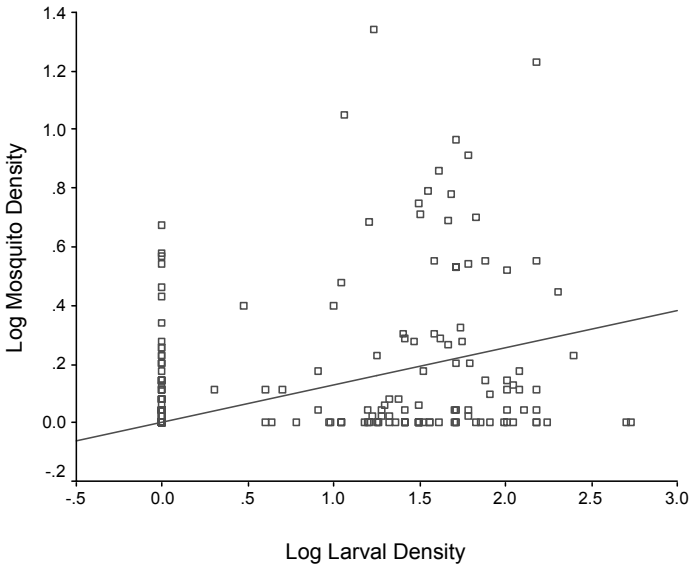


Figure 16. Relationship between larval and adult mosquito densities

3.4. Temporal Distribution Patterns

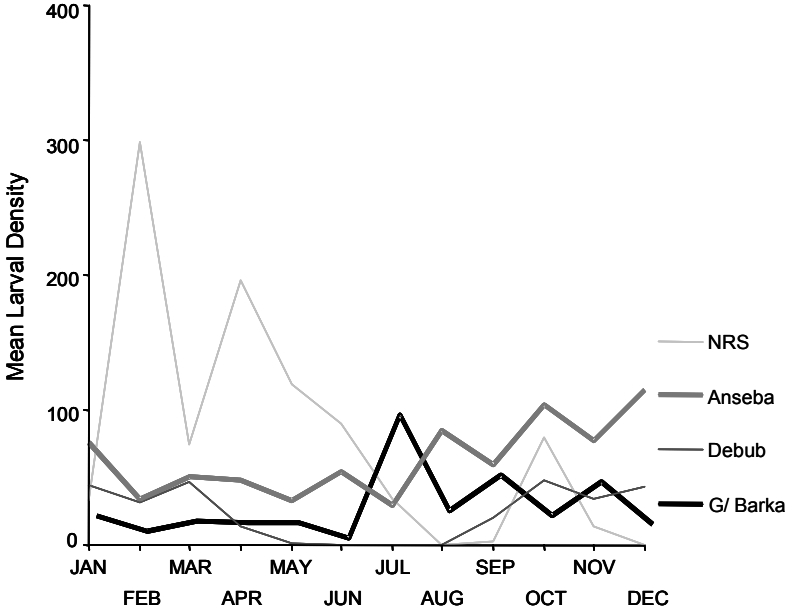
3.4.1. Larval Abundance and Seasonal Patterns

High abundance of anophelines was sampled from Anseba zone (altitude 800–1,600 m above sea level) compared to Debub and Gash-Barka zone. This is an indication of the productivity of the breeding sites in two sites sampled in Anseba zone, and it further stresses the important contribution of streams and rivers in maintaining levels of breeding activity throughout the year in majority of the study sites.

The results also show distinctive temporal or seasonal patterns in larval densities in the study locations (Figure 17 and 18). In all three ecological zones, larval abundance increased when the wet season commenced and decreased in the dry season. The peak densities of larvae were observed at different times and this could be attributed to the different rainfall patterns in the three zones. Of the total anopheline larvae identified to species (n= 2486) using morphological criteria, *An. gambiae*[7], the principal vector of malaria in the country, was predominant and was abundantly sampled in rain pools water channels at communal water supply, and in rivers and streams (Figure 19). In the western lowlands (Gash-Barka), this species was collected in large numbers in the dry season at water supply sites and was abundant in rain pools and on stream pools and edges of streams during the rainy season. A critical finding from the temporal patterns is the fact that breeding of mosquitoes goes on year round, albeit at a generally low level in the dry season. This calls for larval control interventions during the dry and wet seasons.

[7] PCR studies indicate that > 99% of positively identified adult and larval specimens are An. arabiensis. This species is the predominant species of the An. gambiae complex in Eritrea.

A. Fours zones (NRS, Anseba, Gash-Barka and Debub)



B. NRS Excluded

Figure 17. Seasonal patterns of *Anopheles* larval densities by zone

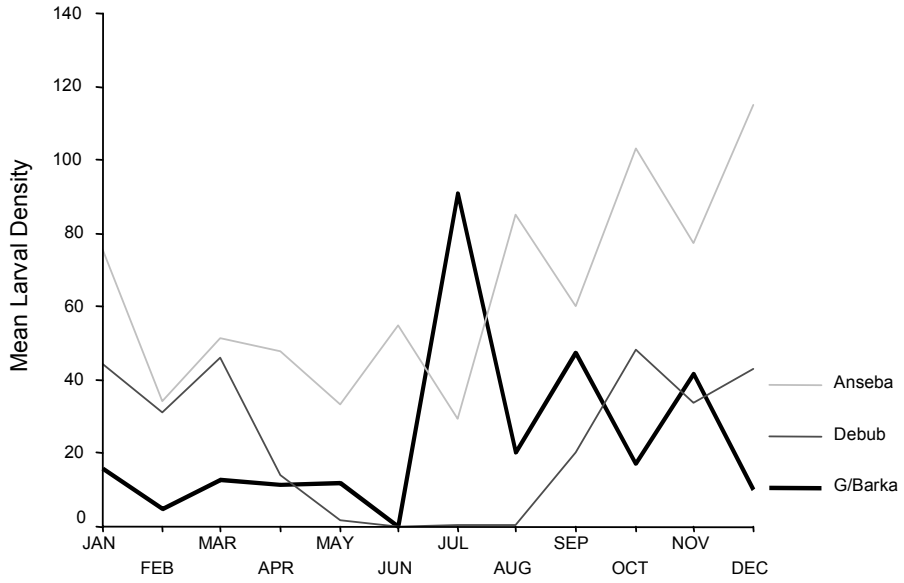


Figure 18. Seasonal patterns of *Anopheles* larval densities by zone

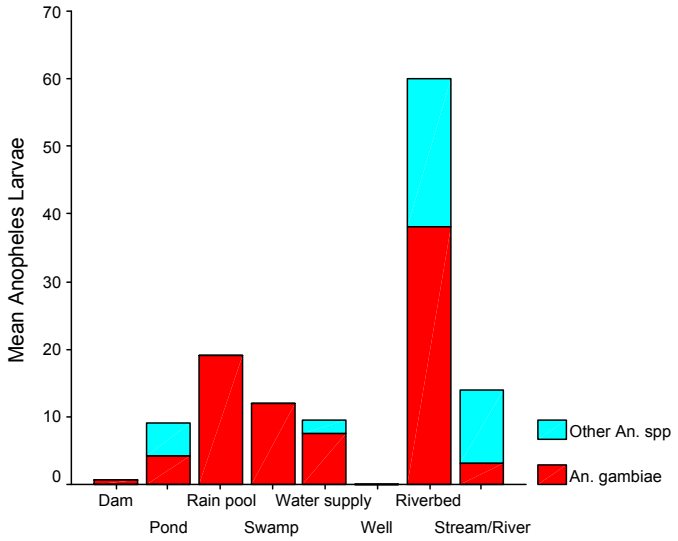


Figure 19. Variation in *Anopheles* larval densities in different breeding habitats
Temporal Distribution of Anopheline Larvae in Different Breeding Habitats

The study demonstrated the relative significance of habitat type on larval productivity in time and space. The interplay of habitat types is shown in Figure 20. It displays the importance of habitat variability by zone and season. While some habitat types were important in one zone, they were either absent or of only low significance in another zone. Notable from the present survey is the fact that breeding activity was maintained year round. This raises the important question of dry season intervention when breeding sites become easier to manage. During the dry season drainage channels at communal water supply points formed the most important larval habitat in the two villages sampled in Gash-Barka zone. This shows the extent to which malaria in the country derives mainly from human modification of the ecosystem. As the country strives toward sustained food sufficiency, a number of dams are under construction. Based on the observations in the present study, such irrigation projects will have a significant impact on malaria transmission through modification of the ecosystem in a way that will substantially support larval breeding. Definitive studies need to be conducted under these situations to elucidate the impact of such schemes on malaria transmission, especially in the lowlands where malaria prevalence is generally focal.

Characteristics of the breeding habitats such as shade, water current, turbidity, and water depth were measured. Three factors—water turbidity, presence of aquatic vegetation and permanence of breeding habitats—were significant in explaining variation in larval densities. Water densities did not seem to vary as a function of temperature ($r = 0.071$, $P = 0.331$). The results generated may not be definitive in explaining variation in larval densities in space and time. A thorough investigation on the chemical analysis of each type of breeding site would be a logical next step to evaluate whether such factors could be used as indicators for the presence and abundance of anopheline larvae over time.

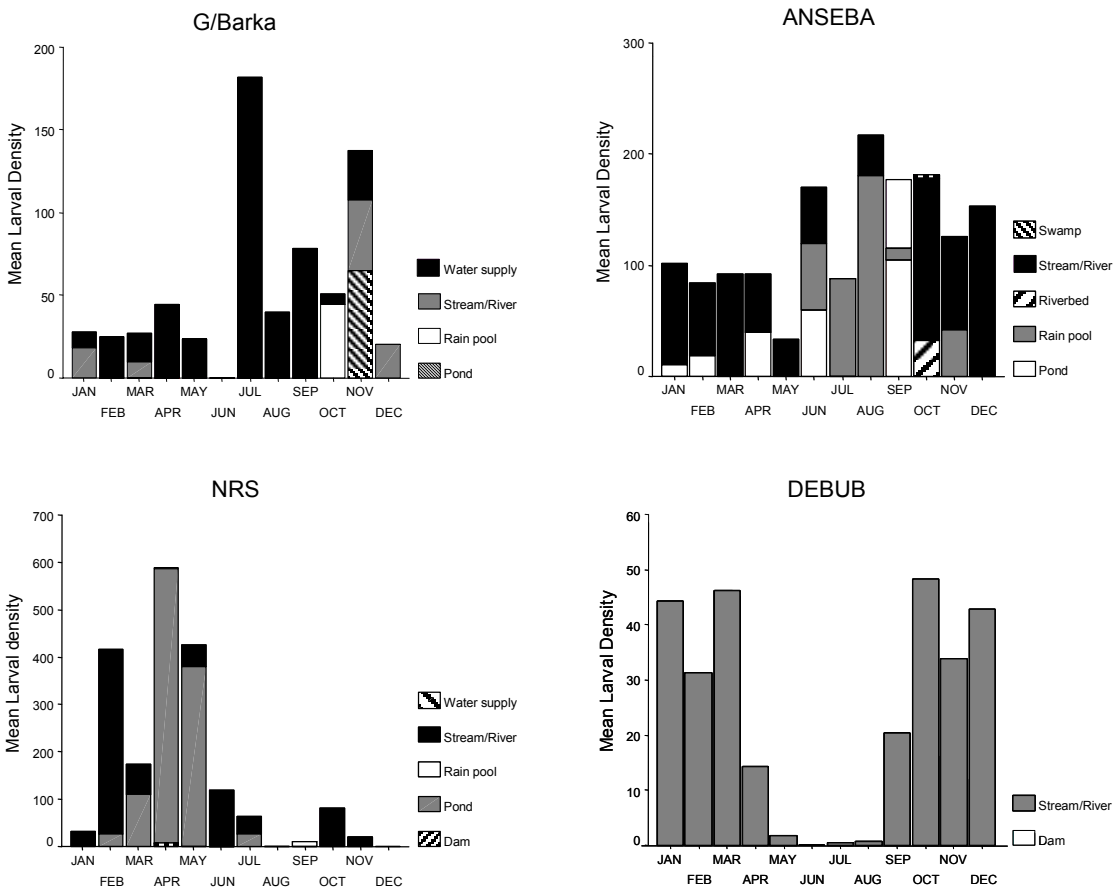


Figure 20. Relative contribution to larval production by different breeding habitats

3.5. Conclusions and Implications for control

- The present study demonstrates the diversity of breeding habitats and their relative significance for *Anopheles* larval production. The breeding sites could be ranked according to larval presence and abundance. This provides a basis for consistent monitoring and targeting of specific breeding sites by use of appropriate larval control strategies on a temporal basis.

- Eight *Anopheles* species were collected with *An. gambiae*[7] predominating in all the positive breeding habitats. Using the vectorial status of the *Anopheles* species as a criterion for implementing larval control would require further information on temporal habitat productivity patterns for the principal vector, *An. gambiae.*

[7]. PCR studies indicate that > 99% of positively identified adult and larval specimens are An. arabiensis. This species is therefore the predominant species of the An. gambiae complex in Eritrea

The results so far suggest the need for implementing larval control measures to all available breeding sites but with a major focus given to the many intermittent streams, river pools and water channels that form the principal anopheline habitats.

- The strong linear association between larval densities and adult mosquito densities suggests that larval control could be a major component of the malaria control program, by checking explosion in adult populations when applied in the wet season (transmission season) and in the dry season as well. The basis for such effective control would rely on active monitoring and subsequent application of larval control measures throughout the year.

- Principal breeding habitats during the dry season include water supply points in communities in the western lowlands where malaria transmission is relatively high. Managing these habitats throughout the year would have an impact on mosquito presence and abundance. Man-made alterations of the ecosystem through irrigation projects need to be independently investigated in light of the present results, as this would have a significant impact on malaria in the country.

3.6. Further Investigation

- Dynamics of habitat productivity to determine the interplay of habitat characteristics in space and time.

- Impact of irrigation schemes on abundance of breeding sites, mosquito densities and malaria transmission.

- Influence of physico-chemical factors and their role as indicators of presence and levels of anopheline production.